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THE ORIGIN AND NATURE OF COLOR IN PLANTS.

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A list of the more important papers published, up until within the past ten years, on the subject of plant colors is given in Dippel's *Das Mikroskop.*[1] Of these the papers by Pringsheim[2] on the examination of chlorophyl and related substances, and by Müller[3] on the spectrum-analysis of the color substances of flowers, are probably the most important.

Pringsheim confined his attention mainly to a spectroscopic study of chlorophyl and the yellow substances in germinating plants, yellow flowers and yellow autumn leaves. He concluded that the yellow substances from these several sources were but modifications of chlorophyl. The yellow principle found in germinating plants he regarded as closely related to chlorophyl, and the yellow substance in autumn leaves as a more remote modification of it. He did not consider, however, as subsequent writers have claimed, that these substances were identical.

Two years before the appearance of Pringsheim's paper, Kraus[4] stated that he had separated from an alcoholic solution of chlorophyl by means of benzol two distinct substances, one yellow and the other blue, the latter being taken up by the benzol. Pringsheim, however, showed that the blue substance was in reality chlorophyl, and that the alcoholic solution, which showed faint chlorophyl-like bands in the spectroscope, still contained some chlorophyl.

While Pringsheim believed that there were two modifications of chlorophyl, one yellow and the other green, the former predominating in germinating plants grown in the dark, and the latter or green substance in leaves exposed to the light, still he did not believe that they could be separated from each other by the method proposed by Kraus.

Yet notwithstanding Pringsheim's well-founded criticisms of the method employed by Kraus, and taking for granted that there were two principles composing chlorophyl, nearly all investigators since Kraus's work was published have practically employed his method as modified by Hansen[5] for the separation of the so-called yellow and green chlorophyl. According to this method of Hansen, fresh material is extracted with 95 per cent. alcohol, the liquid filtered, and to the filtrate 30 to 50 per cent. of water is added; the solution is shaken with petroleum ether and the liquids separated, the ether taking up the green substance, or chlorophyl proper, and the hydro-alcoholic solution holding the yellow principle.

If autumn leaves are treated in the same way, the ether solution will contain very little chlorophyl, while the hydro-alcoholic solution will contain a yellowish or reddish substance, depending upon the kind of material examined. It has usually been considered that this yellow substance in autumn leaves is associated in summer with the active plastids, and on account of its having little food value remains behind. It has furthermore been considered by many that the yellow principle in young leaves is identical with that in autumn leaves and the yellow substance found in yellow flowers, fruits and roots.

Kinds of Colors in Plants.

Colors in plants may be considered to be due to definite constituents which either themselves are colored or produce colors when acted upon by other substances. These substances are found in all parts of the plant, and apparently in all of the cells excepting certain meristematic or dividing cells. They may be divided into two well-differentiated classes, namely, (1) those which are associated with the plastids, or organized bodies in the cell, and (2) those which occur in the cell-sap, or liquid of the cell.

So-called White Colors.

The so-called white colors in plants do not properly belong to either class, but may be said to be appearances rather, due to the absence of color, and depending upon the reflection of light from transparent cells separated by relatively large intercellular spaces containing air. In other words the effect produced by these cells may be likened to that produced by the globules in an emulsion. The white appearance is most pronounced in the pith cells of roots and stems, where on the death of the cells the size of the intercellular spaces is increased and the colorless bodies in the cells as well as the walls reflect the light like snow crystals.

Methods of Extraction.

During this investigation I have examined by means of the Leitz micro-spectroscope the various kinds of coloring substances to which I shall refer but, except in the case of chlorophyl, did not obtain results which were entirely satisfactory, and will endeavor to give special attention to this phase of the subject in another paper. It is frequently difficult to extract and isolate these substances in a sufficiently pure condition for spectroscopic work, particularly as many of them change rapidly.

In this paper, therefore, I shall confine myself to the consideration of the behavior of the extracted coloring substances toward chemical reagents.

The material containing the coloring matter was in all cases separated as nearly as possible from that which was free from color or contained it in less amount. Various solvents were used in the extraction of the coloring substances, depending upon the solubility or nature of the substance. The solvent mostly employed was alcohol (95 per cent.), in some cases dilute alcohol (50 per cent.) or water (hot or cold) was employed.

The plastid colors were extracted by placing the fresh material in 95 per cent. alcohol and allowing it to macerate in the dark for a day or two. I usually took the precaution to tear the material with the fingers rather than to cut it. The solution so obtained contains other than the plastid coloring substances, which latter may be isolated in a more or less pure condition by either of the following methods: (1) The alcohol is distilled off and the solution evaporated on a water bath to near dryness, boiling water is then added and the solution filtered, the extract washed with hot water until the filtrate is colorless; the extract is then taken up with cold alcohol. (2) In the other method the alcoholic solution is diluted with water; and ether, benzin, benzol, xylol, or other similar solvent is added, and the mixture shaken in a separatory funnel. The ethereal layer containing the plastid color may be further purified by shaking it in a separatory funnel with alcohol, adding sufficient water to cause separation of the two layers. The ethereal solution is then distilled and evaporated on a water bath to near dryness, and the pigment taken up with cold alcohol. In either case the alcoholic solution may be boiled for an hour or two with zinc in a reflux condenser, whereby the more or less oxidized plastid pigments are restored. This is a particularly important procedure in the microspectroscopic examination of chlorophyl, and may be used as a means of detecting chlorophyl in other substances.

In order to obtain the coloring principles in early leaves, as the red coloring principle in the leaves of oak, rose, etc., it was found most satisfactory to extract the material with alcohol, add xylol or similar solvent, and then sufficient water to effect separation of the solutions, using a separatory funnel. The cell-sap color remains in the hydro-alcoholic solution, and the traces of xylol should be removed by heating the solution on a water-bath, as the presence of xylol causes a cloudiness in the solution on the addition of the reagents to be subsequently employed.

The cell-sap colors of flowers, as of pansy, tulip, etc., are separated from the plastid pigments in the same way as just mentioned in connection with early leaves.

The cell-sap colors in fall leaves are easily removed by treating the more or less comminuted material with hot or cold water.

In some cases there are several associated colors, and these may be extracted separately by taking advantage of their varying solubility, as in the case of carthamus, where the red principle is extracted with water and the yellow principle with alcohol.

In still other cases special methods are employed, as in the extraction of carotin from carrot according to the method proposed by Husemann.[6] The grated carrot is mixed with water, squeezed through cheese-cloth, and a small quantity of dilute sulphuric acid and tannin added to the mixture, forming a coagulum which settles to the bottom of the precipitating jar. The supernatant liquid is removed by means of a syphon and the coagulum treated six or seven times with 80 per cent. alcohol, which removes mannit and hydro-carotin; the coagulum is then extracted with hot carbon disulphide, which removes the carotin. This solution is evaporated to about half the original volume, an equal amount of absolute alcohol added, and set aside to crystallize, the carotin separating.

One of the striking observations made during this investigation was that in the case of the cell-sap colors the solution was different in color, as compared to the natural color, or sometimes almost colorless, reagents, however, striking colors as intense or even more intense than the original colors.

For the convenience of those who may wish to follow similar studies, the plants which I examined may be grouped according to the solvents which I found best adapted for the extraction of the coloring substances. There is also given the part of the plant employed and the color of the solutions I obtained.

COLOR PRINCIPLES EXTRACTED WITH ALCOHOL.

Name of Plant.	*Part Used.*	*Color of Solution.*
1. Apple (Baldwin) (*Pyrus Malus*)	Epicarp	Light yellowish-red
2. Apple (Bellefleur) (*Pyrus Malus*)	Epicarp	Pale yellow
3. Arbutus (*Epigæa repens*)	Petals	Pale straw
4. Azalea (*Azalea nudiflora*)	Petals	Pale straw
5. Beet (*Beta vulgaris*)	Leaves	Deep green
6. Blackberry (*Rubus Canadensis*)	Stems	Reddish-brown
7. Buttercup (*Ranunculus acris*)	Petals	Deep yellow
8. Cabbage, red (*Brassica oleracea*)	Leaves	Purplish-red
9. Capsicum (*Capsicum fastigiatum*)	Dried fruit	Yellowish-red
10. Carnation, red (*Dianthus Caryophyllus*)	Petals	Deep red
11. Carrot (*Daucus Carota*)	Root	Deep reddish-yellow
12. Celery (*Apium graveolens*)	Etiolated leaves	Bright greenish-yellow
13. Chondrus (*Chondrus crispus*)	Fronds	Light yellowish-green
14. Cinquefoil (*Potentilla Canadensis*)	Petals	Greenish-yellow
15. Cranberry (*Oxycoccus macrocarpus*)	Fruit	Deep red
16. Daffodil (*Narcissus Pseudo-Narcissus*)	Petals	Deep yellow
17. Dandelion (*Taraxacum officinale*)	Petals	Lemon-yellow
18. Dock (*Rumex crispus*)	Spring leaves	Reddish-brown
19. Dogwood (*Cornus Florida*)	Fruit	Brownish-yellow
20. Dulce (*Rhodymenia palmata*)	Fronds	Light yellowish-green
21. Elder (*Sambucus Canadensis*)	Spring leaves	Reddish-brown
22. Fucus (*Fucus vesiculosus*)	Fronds	Greenish-brown
23. Hepatica (*Hepatica triloba*)	Petals	Lemon-yellow or greenish-yellow
23*a*. Hepatica (*Hepatica triloba*)	Involucre	Purplish-red
24. Iris (*Iris versicolor*)	Petals	Violet
25. Jack-in-the-pulpit (*Arisæma triphyllum*)	Spathe	Purplish-red
26. Japanese quince (*Cydonia Japonica*)	Petals	Bright purplish-red
26*a*. Lemon peel	Epicarp	Yellow
27. Mallow (*Malva sylvestris*)	Petals	Violet
28. Maple (*Acer rubrum*)	Flowers	Yellowish or brownish-red
29. Marigold (*Calendula officinalis*)	Petals	Deep yellow
30. Oak, red (*Quercus coccinea*?)	Spring leaves	Reddish-brown
30*a*. Orange peel	Epicarp	Orange-yellow
31. Pansy, blue (*Viola tricolor*)	Petals	Purplish-red

Color Principles Extracted with Alcohol—*Continued.*

Name of Plant.	*Part Used.*	*Color of Solution.*
32. Pansy, yellow (*Viola tricolor*)	Petals	Deep yellow
33. Pineapple (*Ananas sativa*)	Outer portion	Brown
34. Radish (*Raphanus Raphanistrum*)	Purplish layer of root	Light red
35. Rose (*Rosa gallica*)	Dried petals	Light brown
35*a*. Rose (*Rosa* ——)	Early leaves	Reddish-brown
36. Safflower (*Carthamus tinctorius*)	Petals	Deep yellow
37. Saffron (*Crocus sativus*)	Dried stigmas	Yellowish-red
38. Skunk cabbage (*Spathyema fœtida*)	Green leaves	Deep green
39. Skunk cabbage (*Spathyema fœtida*)	Inner portion of leaf buds	Deep yellow
40. Skunk cabbage (*Spathyema fœtida*)	Spathe	Deep yellowish-red
41. Skunk cabbage (*Spathyema fœtida*)	Scales	Purplish-red
42. Skunk cabbage (*Spathyema fœtida*)	Tips of leaf buds	Yellowish-red
43. Spinach (*Spinacea oleracea*)	Leaves	Deep green
44. Sweet Cicely (*Washingtonia Claytoni*)	Spring leaves	Reddish-brown
45. Tomato (*Lycopersicon esculentum*)	Fruit	Pale yellow
46. Tulip (*Tulipa Gesneriana*)	Petals	Light reddish-brown
47. Turnip (*Brassica napus*)	Purplish layer of root	Pale yellow
48. Violet, blue (*Viola cuculata*)	Petals	Pale purplish-red
49. Violet, yellow (*Viola scabriuscula*)	Petals	Yellow
50. Wahoo (*Euonymus Americanus*)	Winter leaves	Reddish-brown

Color Principles Extracted with Dilute Alcohol.

51. Black Mexican corn (*Zea Mays*)	Grains	Light purplish-red
52. Geranium, house (*Pelargonium* ——)	Petals	Light purplish-red
53. Geranium, wild (*Geranium maculatum*)	Petals	Pale straw
54. Houstonia (*Houstonia cærulea*)	Petals	Pale straw
55. Hyacinth, dark red (*Muscari botryoides*)	Petals	Light yellowish-red
56. Hyacinth, blue (*Muscari botryoides*)	Petals	Purplish-red
57. Lilac (*Syringa vulgaris*)	Petals	Brownish-yellow
58. Rhubarb (*Rheum* ——)	Outer portion of petioles	Pale red
59. Strawberry (*Fragaria* ——)	Fruit	Yellowish-red
60. Violet, blue (*Viola cuculata*)	Petals	Greenish-yellow
61. Wistaria (*Kraunhia frutescens*)	Petals	Pale brown

Color Principles Extracted with Water.

62. Beech (*Fagus Americana*)	Autumn leaves	Reddish-yellow
63. Beet (*Beta vulgaris*)	Root	Deep red
64. Blackberry (*Rubus Canadensis*)	Outer portion of stems	Brownish-red
65. Blackberry (*Rubus Canadensis*)	Fruit	Purplish-red
66. Cranberry (*Oxycoccus macrocarpus*)	Fruit	Deep red
67. Dogwood (*Cornus Florida*)	Autumn leaves	Reddish-brown
67*a*. Dulce (*Rhodymenia palmata*)	Fronds	Purplish
68. Elder (*Sambucus Canadensis*)	Dried fruit	Purplish-red
69. Grape (*Vitis vinifera*)	Fruit	Purplish-red
70. Holly (*Ilex Aquifolium*)	Fruit	Deep brownish-red
71. Hydrangea (*Hydrangea Hortensia*)	Neutral flowers	Brownish-red
72. Indian cucumber (*Medeola Virginiana*)	Autumn leaves	Deep brownish-red
73. Mallow (*Malva sylvestris*)	Petals	Dark purplish-red
74. Maple (*Acer saccharum*)	Autumn leaves	Brownish-red
75. Marigold (*Calendula officinalis*)	Dried petals	Deep brownish-red
76. Oak, white (*Quercus alba*)	Autumn leaves	Brownish-red
77. Rhubarb (*Rheum* ——)	Outer portion of petioles	Pale red
78. Rose, wild (*Rosa* ——)	Pericarp	Deep brownish-red
79. Safflower (*Carthamus tinctorius*)	Dried petals	Deep brownish-red
80. Saffron (*Crocus sativus*)	Dried stigmas	Deep yellowish-red
81. Solomon's seal (*Vagnera racemosa*)	Fruit	Deep red

Plastid Color Substances.

The green color in plants is due, as is well known by botanists, to a green pigment known as chlorophyl which is associated with a plastid or organized protoplasmic body, forming a so-called chloroplast. Chlorophyl is distinguished from all other plant substances by possessing a dark broad band between the Fraunhofer lines A and C at the red end of the spectrum, which is apparent even in very dilute solutions. It also shows in more concentrated solutions a broad band extending from F to the violet end of the spectrum, a narrow band between C and D, or the orange portion of the spectrum, and two narrow bands between D and E, or the yellow portion of the spectrum.

Pringsheim examined spectroscopically solutions of the yellow substances found in etiolated germinating leaves, and also the yellow substances of yellow flowers and autumn leaves, and observed the characteristic chlorophyl bands only by using tubes more than three hundred millimeters thick. Inasmuch as small tubes holding five or ten cubic centimeters are sufficient for the examination of chlorophyl, by means of the Zeiss or Leitz microspectroscope, and also because a dilute solution is necessary, one is surprised that Pringsheim and others have used tubes of such enormous thickness, and that they concluded from the more or less indistinct bands which they observed that these substances were modifications of chlorophyl. It is not at all unlikely that what he actually had were concentrated solutions of as many different principles, each of which contained traces of chlorophyl, notwithstanding the care he exercised in separating the green and yellow portions in the material which he used.

In my own studies on the yellow principle of developing leaves I used the buds of skunk cabbage, which develop under ground and under leaves and are of considerable size before exposed to light. The outer light greenish-yellow portions were removed, and only the intense yellow central portion used. This material was extracted in the dark with alcohol. The solution thus obtained is of a pure lemon-yellow color, and may be freed from cell-sap substances either by evaporation to an extract, washing with water, dissolving in cold alcohol, and then boiling with zinc; or by treating the original alcoholic solution with petroleum benzin, whereby the pure yellow leaf substance is separated from the cell-sap substance.

This yellow principle is combined with plastids, which are about one micron in diameter, being spherical or polygonal in shape, and lying closely packed in the palisade cells of both the upper and lower surfaces of the leaf. The yellow plastids are distinguished from the leucoplastids, which occur in the epidermal and mesophyl cells, as well as the chloroplastids, which are found later in the green leaves, by being smaller, relatively more numerous and by not manufacturing either reserve or assimilation starch. The associated pigment is further distinguished from chlorophyl by not being fluorescent; in having a broad band extending from 65 to the red end of the spectrum, and another extending from 50–52 to the violet end of the spectrum, when examined by means of the Leitz micro-spectroscope; and in being less soluble in alcohol and more so in benzin than chlorophyl. This latter characteristic affords a means of partially separating it from chlorophyl, and for this principle I propose the name *etiophyl*, and for the associated plastid, which seems to be a distinct body, I propose a corresponding name, *etioplast*, these terms being used expressly for the purpose of avoiding confusion. The etioplasts completely pack the cells in which they are found, and may be regarded as meristematic plastids, which later give rise to the chloroplastids.

The yellow color in certain roots, flowers and fruits is apparently in all cases due to a yellow pigment associated with a plastid known as a chromoplast. These plastids are distinguished from the other plastids by being of variable shape and in usually containing protein grains. The associated pigment resembles in some respects etiophyl and chlorophyl, in that it is more or less soluble in ether, benzol, xylol, carbon disulphide, etc. These pigments, for the most part, appear to be unaffected by either mineral or organic acids, but usually give some shade of green with alkalies, potassium cyanide, sodium phosphate or iron salts. In some cases they are affected by alum, iodine, sodium nitrite, or sodium nitrite and sulphuric acid, as given in Table I.[1]

[1] In the examination of plant colors the following reagents were found useful: Sulphuric acid, 10 per cent.; hydrochloric acid, 10 per cent.; nitric acid, 10 per cent.; citric acid, 5 per cent.; oxalic acid, 5 per cent.; sodium hydrate, 10 per cent.; ammonium hydrate, 10 per cent.; potassium cyanide, 1 per cent.; sodium phosphate, 5 per cent.; ferric chloride, 3 per cent.; ferrous sulphate, 2.5 per cent.; hydrogen peroxide, 3 per cent.; salicylic acid, saturated solution, gallic acid, 1 per cent.; sodium nitrite, 1 per cent.; sodium nitrite followed by sulphuric

Inasmuch as there seems to be a class of these principles which are distinguished by their solubility, as well as reactions with various chemicals, I venture to propose the name *chromophyl* for these yellowish or orange-colored pigments.

All of the coloring substances given in Table I are soluble in xylol, ether and similar solvents, as well as alcohol, but are sparingly soluble in water.

There are several substances which behave much like the plastid substances, but which are insoluble in xylol, ether, etc., and appear to occupy an intermediate position between the true plastid color substances and the cell-sap colors. I have therefore placed them in class by themselves in Table II.

Cell-sap Color Substances.

During the course of metabolism the plant cell manufactures other color substances which are not combined with the protoplasm, but which are contained in the cell-sap, or liquid of the cell. These substances, unlike the plastid colors, are insoluble in xylol, ether and similar solvents, but are soluble in water and alcohol, which affords a means of separating them from the plastid colors. These cell-sap pigments may occur in cells free from plastids or in the vacuoles of cells containing plastids, but not associated with them as a part of the organized body or plastid. They are usually extracted along with the chlorophyl and remain in the hydro-alcoholic solution after separation of the plastid pigment by means of xylol or other solvent. These pigments have one property in common with the chromophyl substances, namely, with alkalies, potassium cyanide and sodium phosphate, they assume some shade of green. They are distinguished, however, by the fact that the colors are markedly affected by acids and alkalies and by iron salts. They are in most cases also affected by other reagents, as shown in the accompanying tables. These substances being so sensitive to reagents, probably accounts for the various shades and tints characteristic not only of flowers but of leaves as well. My observations on the germinating kernels of black Mexican corn show that even in contiguous cells the constituents associated with the dye

acid; potash alum, 10 per cent.; ammonio-ferric alum, 5 per cent.; iodine solution containing .1 per cent. iodine and 0.5 per cent. potassium iodide; tannin, 3 per cent.

vary to such an extent that the pigment in one cell is colored reddish, in another bluish-green, and in another purplish.

The results of the examination of the cell-sap colors are given in Tables III, IV and V, and while it might seem a very easy matter to divide plant colors into reds, blues and purples, it will be seen that this is almost impracticable, and that the colors given in these tables merge into one another.

An examination of the color substances found in early spring leaves and in autumn leaves showed that these substances are in the nature of cell-sap colors, behaving toward reagents much like the cell-sap colors of flowers, and indeed in some instances they are apparently identical, as will be seen by comparing the results given in Table VI with those given in Tables III, IV and V.

Conclusions.

1. The white appearance in flowers and other parts of plants is due to the reflection and refraction of light in more or less colorless cells separated usually by large intercellular spaces containing air.

2. The green color of plants is due to a distinct pigment, chlorophyl, contained in a chloroplastid, and appears to be more or less constant in composition in all plants. The chloroplastid is furthermore characterized by usually containing starch.

3. The yellow color substance in roots, flowers and fruits is due to a pigment, to which I have given the name chromophyl. This substance is contained in a chromoplastid which varies considerably in shape, and usually contains proteid substances in addition.

4. In the inner protected leaf-buds there is a yellow principle which I have termed etiophyl, and which is contained in an organized body which I have termed an etioplast. The etioplast does not appear to contain either starch or proteid substances.

5. The blue, purple and red color substances in flowers are dissolved in the cell-sap, and are distinguished for the most part from the plastid colors by being insoluble in ether, xylol, benzol, chloroform, carbon disulphide and similar solvents, but soluble in water or alcohol. While quite sensitive to reagents yet none of these colors behave precisely alike.

6. Cell-sap color substances corresponding to the cell-sap colors of flowers are also found in early or spring leaves and in autumn leaves.

In addition I desire to say that I am inclined to look upon the chromoplastids of both flowers and fruits as having the special function of manufacturing or storing nitrogenous food materials, for the use of the developing embryo or developing seed, particularly as protein grains are usually contained in them. The same may be said of the chromoplasts in roots, as in carrot, where the proteids of the chromoplasts are utilized by the plant of the second year.

I am further inclined to consider the cell-sap colors, like other unorganized cell-contents, as alkaloids, volatile oils, etc., to be incident to physiological activity, and of secondary importance in the attraction of insects for the fertilization of the flower and dispersal of the seed.

Finally, I acknowledge my indebtedness to Miss Florence Yaple, Philadelphia, for valuable assistance in the preparation of this paper.

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I. Examination of Plastid Color Substances.

	Etiophyl		Chlorophyl		Chromophyl					
	39. *Skunk cabbage*	12. *Celery*	43. *Spinach*	38. *Skunk cabbage*	16. *Daffodil*	7. *Buttercup*	14. *Cinquefoil*	32. *Yellow pansy*	49. *Yellow violet*	29. *Marigold*
Mineral acids	Cloudy*	Cloudy	Cloudy	Pale brown, cloudy	Cloudy, color less intense	Cloudy	Paler and slightly cloudy	Decolorized, cloudy	Slightly cloudy	No effect
Organic acids	Cloudy	No effect	Cloudy	Light brownish-green	Cloudy, color less intense	Slightly cloudy	Paler and slightly cloudy	Decolorized, cloudy	Slightly cloudy	No effect
Alkalies	O.c.† intensified	Yellowish-green	No effect	No effect	Slightly green	No effect	Yellowish-green	Yellowish-green	Yel'sh-green, becoming colorless	Light yellowish-green
Potassium cyanide	O. c. intensified	Yellowish-green	No effect	No effect	Slightly green	No effect	Yellowish-green	Yellowish-green	Yellowish-green	Light yellowish-green
Sodium phosphate	O. c. intensified	Yellowish-green	No effect	No effect	Slightly green	No effect	Yellowish-green	Yellowish-green	Yellowish-green	Light yellowish-green
Ferric chloride	Light olive-green	Brown	No effect	Brownish-green	Light olive-gr'n, slightly cloudy	Yellowish-brown, cloudy	Brownish-green	Olive-green	Yellowish-green	Greenish-brown
Ferrous sulphate	Light olive-green, cloudy	Pale brown	Light brown	Brownish-green	Light olive-gr'n, slightly cloudy	Yellowish-green, cloudy	Pale green	Green, becoming olive-green	Yellowish-green, cloudy	Pale greenish-brown
Salicylic acid	Remains clear	No effect	No effect	No effect	No effect	No effect	Slightly decolorized	Decolorized	Partly decolorized	No effect
Gallic acid	Remains clear	No effect	No effect	No effect	No effect	No effect	Slightly decolorized	Decolorized	Partly decolorized	No effect
Hydrogen peroxide	No effect	No effect	No effect	No effect	No effect	No effect	Slightly decolorized	Decolorized	Partly decolorized	No effect
Sodium nitrite	No effect	No effect	No effect	No effect	No effect	No effect	No effect	No effect	Faint brown	No effect
Sodium nitrite and sulphuric acid	Cloudy	Cloudy	Light bluish-green	Pale brown, cloudy	Decolorized	Decolorized	Decolorized	Faint brown	Decolorized	No effect
Alum	Cloudy	Slightly cloudy	Cloudy	Pale brownish-green, cloudy	Cloudy	Cloudy	No effect	Pale green, cloudy	Pale green, cloudy	No effect
Ammonio-ferric alum	Olive-green	Brown	Greenish-brown	Brownish-green, cloudy	Olive-green	Yellowish-brown	Gr'n-brown, changing to brown	Brownish-green	Pale yellowish-brown	Greenish-brown
Iodine solution	No effect	No effect	No effect	Greenish-brown	Slightly cloudy	Pure green, distinct	No effect	Brownish	No effect	No effect
Tannin	No effect	No effect	No effect	No effect	Slightly cloudy	No effect	No effect	Slightly decolorized	No effect	No effect

*The cloudy appearance of solutions recorded in this table is probably due to an oily or resinous substance associated with the coloring principle, or to the fact that the coloring principle is insoluble in much diluted alcohol.

† O. c., original color of solution.

I. Examination of Plastid Color Substances—*Continued.*

	Chromophyl								
	75. *Marigold*	17. *Dandelion*	46. *Tulip*	26a. *Lemon peel*	30a. *Orange peel*	45. *Tomato*	11. *Carrot*	25. *Jack-in-the-pulpit*	78. *Wild rose hips*
Mineral acids . . .	No effect	Cloudy	Slightly cloudy	No effect	No effect	Cloudy	Cloudy	No effect	Slightly cloudy
Organic acids . . .	No effect	Slightly cloudy	Slightly cloudy	No effect	No effect	Cloudy	Slightly cloudy	No effect	Slightly cloudy
Alkalies	O. c. slightly intensified	No effect	Faint yellowish-green	Pure yellow	Greenish-yellow	Very faint green	Slightly greenish	No effect	No effect
Potassium cyanide	O. c. slightly intensified	No effect	Faint yellowish-green	Pure yellow	Greenish-yellow	Very faint green	Slightly greenish	No effect	No effect
Sodium phosphate	O. c. slightly intensified	No effect	Faint yellowish-green	Pure yellow	Light greenish-yellow	Very faint green	Slightly greenish	No effect	No effect
Ferric chloride . .	Olive-green, changing to brown	Greenish-yellow	Greenish-yellow	Dark brown	Yellowish-brown	Yellowish-green	Very light brown	No effect	Light greenish-brown
Ferrous sulphate .	Pale olive-green	Cloudy, pale yellowish-green	Faint green	Dark brown	Greenish-yellow	Pale green	No effect	No effect	Light greenish-brown
Salicylic acid . . .	No effect	No effect	No effect	No effect	No effect	No effect	No effect	No effect	No effect
Gallic acid	No effect	No effect	No effect	No effect	No effect	No effect	No effect	No effect	No effect
Hydrogen peroxide	Pale yellow	No effect	No effect	No effect	No effect	No effect	No effect	No effect	No effect
Sodium nitrite. . .	Pale yellow	Slightly cloudy	No effect	No effect	No effect	Decolorized	No effect	No effect	No effect
Sodium nitrite and sulphuric acid. .	No effect	Decolorized	Decolorized	Light brown	No effect	Decolorized	Decolorized, bluish fluorescence	Decolorized	Decolorized
Alum	No effect	Slightly cloudy	No effect	Pale green	No effect	Decolorized	Slightly cloudy	No effect	No effect
Ammonio-ferric alum	Dark yellowish-brown	Greenish-yellow, cloudy	Brownish-yellow	Deep greenish-brown	Light yellowish-brown	Yellowish-brown	No effect	No effect	Light greenish-brown
Iodine solution . .	No effect	No effect	No effect	No effect	No effect	Faint green	Pure green, or yellow with green fluorescence	No effect	No effect
Tannin	No effect	No effect	No effect	No effect	No effect	No effect	No effect	No effect	No effect

II. Examination of Intermediate Color Substances.

	37, 80. *Saffron*	36. *Safflower*	79. *Safflower*	2. *Apple (Bellefleur)**	70. *Holly*
Mineral acids	No effect	Cloudy	No effect	No effect	Light yellow
Organic acids	No effect	Cloudy	No effect	No effect	Slightly decolorized
Alkalies	No effect	Greenish-yellow	Darkened slightly	Greenish-yellow	Brown
Potassium cyanide	No effect	Greenish-yellow	Darkened slightly	Greenish-yellow	No effect
Sodium phosphate	No effect	Greenish-yellow	Darkened slightly	Pale yellow	No effect
Ferric chloride	Darkened or greenish-brown	Light olive-green to light brown	Dark greenish-brown	Green, changing to olive-green	Light greenish-brown
Ferrous sulphate	No effect	Light olive-green to light brown	Light greenish-brown	Pale green	Light greenish-brown
Salicylic acid	No effect	No effect	No effect	No effect	No effect
Gallic acid	No effect	No effect	No effect	No effect	No effect
Hydrogen peroxide	No effect	No effect	No effect	No effect	No effect
Sodium nitrite	No effect	No effect	No effect	Pale brown	No effect
Sodium nitrite followed by sulphuric acid	Pale yellow	Cloudy	No effect	Light brown	No effect
Alum	No effect	No effect	No effect	No effect	No effect
Ammonio-ferric alum	Darkened or yellowish-brown	Light yellowish-brown	Deep olive-brown	Greenish to greenish-brown	Greenish-brown
Iodine solution	No effect	No effect	No effect	No effect	No effect
Tannin	No effect	No effect	No effect	No effect	No effect

* Cloudy on addition of water to alcoholic solution.

III. EXAMINATION OF BLUE CELL-SAP COLOR SUBSTANCES.

	23. *Hepatica*	48. *Violet, blue*	31. *Pansy, blue*	56. *Hyacinth, blue*	61. *Wistaria*	54. *Houstonia*	27. *Mallow flowers*	*Litmus solution*	24. *Iris*
Natural color	Blue	Violet-blue	Purple	Purplish-blue	Light blue	Light blue	Dark blue	Deep purple	Purple to violet
Mineral acids	Pale yellowish-red	Pure deep red	Intense rich red	Intense rich red	Purplish-red	Light yellowish-red	Deep purplish-red	Yellowish-red	Pure deep red
Organic acids	Pale yellowish-red	Pure red	Purplish-red	Violet-red	Pale purplish-red	Light yellowish-red	Deep purplish-red	Yellowish-red	Pure deep red
Alkalies	Green	Green	Green to brownish-green	Light brownish-green	Yellowish-green	Yellowish-green	Brownish-green	Pure blue	Green, changing to yellowish-green
Potassium cyanide	Green	Green	Green	Green	Yellowish-green	Yellowish-green	Brownish-green	Pure blue	Green
Sodium phosphate	Pale green	Green	Green	Green	Yellowish-green	Yellowish-green	Green	Purplish-blue	Green
Ferric chloride	Olive-green	Olive-green	Intense blue	Purplish-brown to brown	Olive-green	Deep olive-green	Brownish-green	Purplish-red	Purplish-blue, changing to brown
Ferrous sulphate	Light olive-green	Bluish-green	Deep blue	Blue	Brownish-purple	Olive-green	Reddish-brown	Purplish-red	Pure blue
Salicylic acid	Faint yellowish-red	Faint red	O. c. intensified	No effect	Pale reddish	Slightly reddened	No change	Yellowish-red	Faint red
Gallic acid	Faint yellowish-red	Slight red	O. c. intensified	O. c. intensified	No effect	No effect	No effect	Yellowish-red	Faint red
Hydrogen peroxide	No effect	Slight red	O. c. intensified	No effect	No effect	No effect	No effect	Yellowish-red	No effect
Sodium nitrite	No effect	Green	Pure green	No effect	Slightly greenish	Light green	Pale purplish	No effect	Decolorized
Sodium nitrite, followed with sulphuric acid	Pale yellowish-brown	Red, becoming decolorized	Red, then colorless	Yellowish-red	Pale reddish	Light yellowish-red	Golden yellow	Yellowish-red	Faint red, almost decolorized
Alum	Slightly yellowish-gr'n	Gobelin-blue	Sky-blue, light blue	Decolorized	No effect	No effect	No effect	Yellowish-red	Pure blue, distinct
Ammonio-ferric alum	Olive-green	Greenish-brown	Deep blue, rapidly changing to bluish-gr'n	Reddish-brown	Olive-green	Olive-green	Greenish-brown	Yellowish-red	Purplish, changing to brown
Iodine and potassium iodide	No effect	No effect	Pale yellowish-red	No effect	No effect	No effect	No effect	Blue	Decolorized
Tannin	No effect	No effect	Reddened	No effect	No effect	No effect	No effect	Purplish-red	No effect

IV. Examination of Purple Cell-sap Color Substances.

	25. *Jack-in-the-pulpit*	40. *Skunk cabbage*	41. *Skunk cabbage*	23a. *Hepatica involucres*	57. *Lilac*	51. *Black Mexican corn*	68. *Elderberries*
Natural color	Violet-red	Purplish-red	Purplish-red	Purplish	Purple	Purplish	Purplish
Mineral acids	Pure deep red	Deep red	Red	Faint salmon	Somewhat cloudy	Pure red	Purplish-red
Organic acids	Pure deep red	Light purplish-red	Light red	Faint salmon	Somewhat cloudy	Pure red	Purplish-red
Alkalies	Green	Intense green	Green	Yellowish-green	Greenish, changing to yellowish-brown	Bluish-green	Pure green
Potassium cyanide	Green	Intense green	Green	Yellowish-green	Greenish, changing to yellowish-gr'n	Bluish-green	Pure green
Sodium phosphate	Green	Green	Green	Pale yellowish-green	Greenish, changing to yellowish-gr'n	Light bluish-green	Light green
Ferric chloride	Purplish-red, changing to brown	Dark purple	Purplish-red	Pale greenish-brown	Deep brownish-green	Greenish-brown	Pale greenish-brown
Ferrous sulphate	Violet	Dark purple	Purplish-red	Very pale greenish-brown	Faint olive-green	Purple	Pale purplish
Salicylic acid	Pure red	No effect	No effect	No effect	No effect	Pinkish	O. c. slightly intensified
Gallic acid	Pure red	No effect	No effect	No effect	No effect	Slightly pink	O. c. slightly intensified
Hydrogen peroxide	Pure red	No effect	No effect	No effect	No effect	Red	No effect
Sodium nitrite	No effect	No effect	Becoming cloudy	No effect	No effect	No effect	Pale brown
Sodium nitrite, followed by sulphuric acid	Pure deep red	Yellowish-red	Yellowish-red or orange	Pale yellowish-brown	Pale yellow	Yellowish-red	Purplish-red
Alum	Violet	Purplish-red, fluorescent	Faint purplish-red	No effect	No effect	Red, changing to violet	No effect
Ammonio-ferric alum	Purplish-brown	Greenish-brown	Purplish-green	Greenish-brown	Deep brownish-green	Yellowish-brown	Olive-green
Iodine solution	No effect	No effect	No effect	No effect	No effect	No effect	No effect
Tannin	Slightly red	No effect	No effect	No effect	No effect	Faint pink	No effect

IV. EXAMINATION OF PURPLE CELL-SAP COLOR SUBSTANCES—*Continued.*

	64. *Blackberry*	69. *Concord grapes*	8. *Red cabbage*	47. *Turnip*	71. *Hydrangea*	67a. *Dulce*	53. *Wild geranium*
Natural color . . .	Reddish-purple	Bluish-purple	Purplish-red	Purplish-red	Reddish-purple	Purplish-red	Light purplish-red
Mineral acids . . .	Purplish-red	O. c. intensified	Rose-red	Purplish-red	Yellowish-red	Purple, losing fluorescence	Deep red
Organic acids . .	Purplish-red	O. c. intensified	Light rose-red	Light purplish-red	Yellowish-red	Purple, losing fluorescence	Faint red
Alkalies	Brownish-purple	Pure green, changing to olive-green	Intense green	Light green	Yellowish-green	Pale yellowish-brown	Yellowish-green, changing to yellowish-brown
Potassium cyanide.	Brownish-purple	Pale bluish-green	Intense green	Light green	Yellowish-green	Yellowish-brown	Greenish-yellow
Sodium phosphate .	Slightly changed	Violet	Bluish-green	Light green	Yellowish-green	No effect	Greenish-yellow
Ferric chloride . . .	Purplish-brown, changing to brown	Greenish-brown	Rose-purple	Light greenish-brown	Olive-green	Purplish-brown	Deep olive-green
Ferrous sulphate . .	Purple	Purplish-brown	Purple	Light blue	Olive-green	No effect	Blue
Salicylic acid	No effect	No effect	O. c. intensified	No effect	Slightly redden'd	No effect	Red
Gallic acid	No effect	No effect	O. c. intensified	Slightly pink	Slightly redden'd	Slightly purple	No effect
Hydrogen peroxide	No effect	No effect	No effect	No effect	No effect	No effect	No effect
Sodium nitrite . . .	Purple color intensified	Purplish-brown	No effect	No effect	Light greenish	No effect	No effect
Sodium nitrite, followed by sulphuric acid	Brownish-red	Brown	Yellowish-red	Pale yellowish to brownish-red	Brownish-yellow	Faint purple, losing fluorescence	Deep red
Alum	No effect	No effect	Violet, purplish-red	Faint violet	No effect	No effect	No effect
Ammonio-ferric alum	Deep purple	Olive-green	Purplish-brown	Pale brown	Olive-green	Purplish-brownish red	Bluish-brown green
Iodine solution .	No effect	No effect	No effect	No effect	No effect	No effect	Red, becoming colorless
Tannin	No effect	No effect	No effect	No effect	No effect	O.c. rendered bluish	No effect

V. Examination of Red Cell-sap Color Substances.

	52. *House geranium*	46. *Tulip*	10. *Carnation*	55. *Hyacinth*	35. *Rose*	4. *Azalea*	28. *Maple flowers*	15. *Cranberry*
Natural color . . .	Very deep red	Reddish	Deep red	Red	Red	Pink	Red	Deep red
Mineral acids . . .	Deep orange-red	Purplish-red	Yellowish-red	Deep orange-red	Rose red	Pale red	Yellowish-red, cloudy	No change
Organic acids . . .	Pale orange-red	Purplish-red	O. c. intensified	O. c. intensified	Light purplish-red	Pale red	Yellowish-red, clear	No change
Alkalies	Yellowish-green, changing to brown	Green	Olive-green, changing to red'sh-brown	Green, changing to brown	Green, changing to brown	Light greenish-yellow	Dark green	Brownish-purple
Potassium cyanide.	Yellowish-green	Green	Olive green	Green	Green	Light greenish-yellow	Green, changing to brown	Brownish-purple
Sodium phosphate.	No effect	Light green	Light green	Green	Green	Pale greenish-yellow	Yellowish-green	Brownish-purple
Ferric chloride . .	Purplish, changing to greenish brown	Purplish-br'wn to brown	Brownish-red	Deep red	Greenish-black	Greenish-yellow	Olive-green	Brownish-red
Ferrous sulphate .	Deep violet	Deep brownish-red	Brownish-red	Pinkish changing to wine-red	Bluish-black	Pale purplish-brown	Deep blue	Reddish-brown
Salicylic acid . . .	Yellowish-red	Purplish-red	O. c. intensified	O. c. intensified	O. c. intensified	Faint pink	Yellowish-red	No effect
Gallic acid.	Yellowish-red	Purplish-red	O. c. intensified	O. c. intensified	O. e. intensified	Faint pink	Yellowish-red	No effect
Hydrogen peroxide	Yellowish-red	Light purplish-red	O. c. intensified	O. c. intensified	O. c. intensified	No effect	Faint yellowish-red	No effect
Sodium nitrite . .	No effect	Light brownish-red	Slightly decolorized	No effect	Slightly decolorized	No effect	Light greenish-yellow	Yellowish-brown
Sodium nitrite, followed with sulphuric acid . . .	Deep orange-red	Light brown	O. c. restored and intensified	Deep orange-red	Yellowish-red	Pale red	Yellowish-red, cloudy	Yellowish-brown
Alum	Pale red	Purplish-red	Yellowish-red	Yellowish-red	Pale yellow, changing to reddish-brown with a greenish fluorescence	Faint pink	Purplish-red, cloudy	No effect
Ammonio-ferric alum	Deep violet, changing to purplish-brown	Brown	Brownish-red	Deep red	Dark olive-green	Pale greenish-brown	Deep blue	Brown
Iodine solution . .	Red, becoming decolorized	No effect	No effect	No effect	Yellowish-red	No effect	No effect	No effect
Tannin	Faint red	Purplish-red	No effect	No effect	No effect	No effect	No effect	No effect

V. EXAMINATION OF RED CELL-SAP COLOR SUBSTANCES—*Continued.*

	59. *Strawberry*	81. *Solomon's seal*	58, 77. *Rhubarb*	34. *Radish*	63. *Beet*	1. *Baldwin apple*	33. *Pineapple*
Natural color	Yellowish-red to red	Ruby red	Pinkish to red	Bright red	Deep red	Red	Slightly reddened
Mineral acids	O. c. intensified	O. c. intensified	O. c. much intensified	Yellowish-red	No effect	O. c. intensified	Apparently no effect
Organic acids	O. c. slightly intensified	O. c. intensified	O. c. intensified	O. c. much intensified	No effect	O. c. somewhat intensified	Pale yellowish-green
Alkalies	Brownish	Greenish-brown	Light green	Green	Green	Greenish-yellow	Pale yellowish-green
Potassium cyanide	Purplish	Greenish-brown	Light green	Slight blue	Green	Greenish-yellow	Apparently no effect
Sodium phosphate	Pale purple	Greenish-brown	Almost decolorized	Slight blue, then decolorized	Greenish	Yellowish-green	Yellowish-green
Ferric chloride	Reddish-brown	Reddish-brown	Brownish	Red	Greenish-brown	Deep green	Pale yellowish-green
Ferrous sulphate	Brownish-red	Light reddish-brown	Reddish	Red, slightly cloudy	Light greenish-brown	Brownish-green	No effect
Salicylic acid	No effect	No effect	No effect	O. c. intensified	No effect	No effect	No effect
Gallic acid	No effect	No effect	No effect	O. c. strongly intensified	No effect	No effect	No effect
Hydrogen peroxide	No effect	No effect	No effect	O. c. intensified	No effect	No effect	No effect
Sodium nitrite	Slightly brown	No effect	Decolorized	No effect	No effect	No effect	No effect
Sodium nitrite, followed with sulphuric acid	Pale yellowish-brown	Light reddish-brown	Pale yellow	Bright red	No effect	Brownish-red or brick-red	Light brown
Alum	O. c. intensified	No effect	Purplish-red	Pale yellowish-red	No effect	No effect	No effect
Ammonio-ferric alum	Brown	Brownish-red	Pale brown	Deep yellow or yellowish-brown	Dark brown	Olive-green	Pale greenish-brown
Iodine solution	No effect	No effect	No effect	No effect	No effect	No effect	No effect
Tannin	No effect	No effect	No effect	No effect	No effect	No effect	No effect

VI. Examination of Leaf Coloring Principles.

	35. *Rose*	42. *Skunk cabbage*	30. *Oak*	6. *Blackberry*	21. *Elder*	18. *Dock*	50. *Wahoo*
Natural color	Greenish-red	Purplish-red	Deep red	Brownish-red	Greenish-red	Greenish-red	Greenish-red
Mineral acids	Rose color	Yellowish-red	Orange-red	Pale brownish-yellow	Light purplish-red	Slightly reddish	Yellowish-red
Organic acids	Rose color	Light yellowish-red	Orange-red	Pale brownish-yellow	Pale yellowish-red	No effect	Slightly reddened
Alkalies	Green, changing to greenish-yellow	Green	Greenish-brown	O. c. intensified	Intense yellowish-green	Yellowish-green	Greenish
Potassium cyanide	Pale green	Green	Greenish-brown	O. c. intensified	Intense green	Yellowish-green	Brownish-green
Sodium phosphate	Pale greenish-yellow	Green	Pale greenish-brown	O. c. slightly intensified	Intense green	Yellowish-green	Yellowish-green
Ferric chloride	Olive-green	Dark brownish-green	Bluish, changing to purplish	Olive-green, changing to brown	Deep green	Dark green	Olive-green
Ferrous sulphate	Deep blue	Dark brownish-green	Indigo blue	Pale olive-green	Deep green	Dark green	Faint orange-green
Salicylic acid	Pale red	No effect	Slightly orange-red	No effect	Faint yellowish-red	No effect	No effect
Gallic acid	Pale red	No effect	Slightly orange-red	No effect	Faint yellowish-red	No effect	No effect
Hydrogen peroxide	No effect	No effect	Slightly orange-red	No effect	Faint yellowish-red.	No effect	No effect
Sodium nitrite	No effect	No effect	No effect	No effect	Slightly green	No effect	Slightly green
Sodium nitrite, followed with sulphuric acid	Yellowish-brown	Brownish-yellow	Reddish-brown	No effect	Reddish, changing to brown	*Deep brownish-red*	Yellowish-red
Alum	Reddish-brown	No effect	Purplish or violet colored	No effect	Pale yellowish-brown	Pale yellowish-green	No effect
Ammonio-ferric alum	Olive-green	Purplish-green	Bluish-brown	Brownish-green	Deep green	Dark green	Olive-green
Iodine solution	No effect	No effect	Green, changing to purplish-brown	No effect	No effect	No effect	No effect
Tannin	Faint purplish-red	No effect	No effect	No effect	No effect	No effect	No effect

VI. EXAMINATION OF LEAF COLORING PRINCIPLES—*Continued.*

	44. *Sweet cicely*	74. *Maple*	76. *Oak*	67. *Dogwood*	72. *Indian cucumber*	62. *Beech.*
Natural color	Greenish-red	Dark red	Dark red	Dark red	Reddish	Greenish to brownish-yellow
Mineral acids	Faint yellowish-red	Yellowish-red	Yellowish-red	Yellowish-red	Deep purplish-red	Partly decolorized
Organic acids	Faint yellowish-red	Slightly yellowish-red	Slightly yellowish-red	Yellowish-red	Deep purplish-red	Partly decolorized
Alkalies	Yellowish	Olive-green, with ammonia, reddish brown	Reddish-brown	Brown	Green	Brown
Potassium cyanide	Yellowish	Deep brownish-red	Purplish-red	Brown	Green	Very light brown
Sodium phosphate	Yellowish	Light olive-green	Greenish-brown	Light brown	Light green	Faint brown
Ferric chloride	Olive-green	Deep blue precipitate	Blue, changing rapidly to olive-green	Deep blue, changing to olive-green	Reddish-brown	Greenish-brown
Ferrous sulphate	Olive-green	Deep blue solution	Blue	Deep blue	Reddish-brown	Pale greenish-brown
Salicylic acid	No effect	Slightly yellowish-red	No effect	Yellowish-red	Purplish-red	Partly decolorized
Gallic acid	No effect	Slight effect	No effect	Yellowish-red	Purplish-red	Partly decolorized
Hydrogen peroxide	No effect	No effect	No effect	No effect	Purplish-red	Partly decolorized
Sodium nitrite	Brownish	No effect	No effect	Brownish-yellow	Faint brown	No effect
Sodium nitrite, followed with sulphuric acid	Yellowish-brown	Yellowish-red	Yellowish-red	Yellowish-red, changing to yellowish-brown	Faint yellowish-brown	Light brown
Alum	Light greenish-yellow	No effect	No effect	Purplish-red	Purplish-red	Partly decolorized
Ammonio-ferric alum	Olive-green	Deep blue	Blue, changing to olive-green	Deep blue, changing to olive-green	Purplish-brown	Greenish-brown
Iodine solution	No effect	No effect	No effect	No effect	No effect	No effect
Tannin	No effect	No effect	No effect	No effect	Purplish-red	No effect